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NEWS 7 MAY 19 Derwent World Patents Index to be reloaded and enhanced
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                      USPATFULL/USPAT2
NEWS 9 MAY 30
                      The F-Term thesaurus is now available in CA/CAplus
NEWS 10 JUN 02 The first reclassification of IPC codes now complete in
                      INPADOC
NEWS 11 JUN 26 TULSA/TULSA2 reloaded and enhanced with new search and
                      and display fields
NEWS 12 JUN 28 Price changes in full-text patent databases EPFULL and PCTFULL
NEWS 13
           JUl 07
                      Coverage of Research Disclosure reinstated in DWPI
NEWS 14 JUl 11 CHEMSAFE reloaded and enhanced
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FILE 'HOME' ENTERED AT 15:13:36 ON 13 JUL 2006

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- => (fluorescen? (s) fluctuat?) or fluorescence (w) correlation L1 6474 (FLUORESCEN? (S) FLUCTUAT?) OR FLUORESCENCE (W) CORRELATION
- => pathogen and 11

L2 27 PATHOGEN AND L1

=> dup rem 12

PROCESSING COMPLETED FOR L2

L3 22 DUP REM L2 (5 DUPLICATES REMOVED)

=> py>2002

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=> py>2002 and 13

L4 14 PY>2002 AND L3

- => t ti 13 1-22
- L3 ANSWER 1 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Detection of prion particles in samples of BSE and scrapie by fluorescence correlation spectroscopy without proteinase K digestion.
- L3 ANSWER 2 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN
- TI Antibody and fluorescently labeled antibody fragment specific to different epitopes of same antigen for immunodetection of prion or food antigen
- L3 ANSWER 3 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Disassembly of structurally modified viral nanoparticles: characterization by fluorescence correlation spectroscopy.
- L3 ANSWER 4 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Molecular and structural characterization of fluorescent human parvovirus B19 virus-like particles.
- L3 ANSWER 5 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Membrane perturbation effects of peptides derived from the N-termini of

unprocessed prion proteins.

- L3 ANSWER 6 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Measuring protein-protein interactions inside living cells using single color fluorescence correlation spectroscopy.

  Application to human immunodeficiency virus type I integrase and LEDGF/p75.
- L3 ANSWER 7 OF 22 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
- TI Method of measuring molecular interactions
- L3 ANSWER 8 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Effect of metal ions on de novo aggregation of full-length prion protein.
- L3 ANSWER 9 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Assembly of fluorescent chimeric virus-like particles of canine parvovirus in insect cells.
- L3 ANSWER 10 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI HIV-1 nucleocapsid protein binds to the viral DNA initiation sequences and chaperones their kissing interactions.
- L3 ANSWER 11 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Observation of a fluorescently labelled virus on its infectious entry pathway into the cell using Fluorescence Correlation Spectroscopy.
- L3 ANSWER 12 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Monitoring human parvovirus B19 virus-like particles and antibody complexes in solution by fluorescence correlation spectroscopy.
- L3 ANSWER 13 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Direct measurement of Gag-Gag interaction during retrovirus assembly with FRET and fluorescence correlation spectroscopy.
- L3 ANSWER 14 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Destabilization of the HIV-1 complementary sequence of TAR by the nucleocapsid protein through activation of conformational fluctuations.
- L3 ANSWER 15 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Molecular diagnostics of transmissible spongiform encephalopathies.
- L3 ANSWER 16 OF 22 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
- TI Species-specific detection of organisms, particularly for identifying bacteria causing sepsis, comprises amplification and elongation of ribosomal nucleic acid.
- L3 ANSWER 17 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Fluorescence correlation spectroscopy for in vivo monitoring of resistance mechanisms.
- L3 ANSWER 18 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Putting prions into focus: Application of single molecule detection to the

diagnosis of prion diseases.

- L3 ANSWER 19 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Rapid and reproducible quantification of hepatitis C virus cDNA by fluorescence correlation spectroscopy.
- L3 ANSWER 20 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Fluorescence correlation spectroscopy for rapid and sensitive HCV RNA quantification.
- L3 ANSWER 21 OF 22 MEDLINE on STN DUPLICATE 2
- TI Fluorescence correlation analysis of probe diffusion simplifies quantitative pathogen detection by PCR.
- L3 ANSWER 22 OF 22 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
- TI Fluorescence correlations, single molecule detection and large number screening. Applications in biotechnology.

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=> e wolf david?/au
                   WOLF DAVID S/AU
E1
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     (FILE 'HOME' ENTERED AT 15:13:36 ON 13 JUL 2006)
     FILE 'MEDLINE, BIOSIS, CAPLUS, SCISEARCH, EMBASE, WPIDS' ENTERED AT
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L1
           6474 (FLUORESCEN? (S) FLUCTUAT?) OR FLUORESCENCE (W) CORRELATION
             27 PATHOGEN AND L1
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             22 DUP REM L2 (5 DUPLICATES REMOVED)
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L4 14 PY>2002 AND L3 E WOLF DAVID?/AU

E WOLF DAVID E?/AU

L5 73 E2 OR E4

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7 L5 AND L1

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21 L3 NOT L7

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ANSWER 1 OF 7 MEDLINE on STN

ACCESSION NUMBER: 2004022450 MEDLINE DOCUMENT NUMBER: PubMed ID: 14719345

TITLE: Fluorescence correlation spectroscopy:

molecular complexing in solution and in living cells.

AUTHOR: Bulseco Dylan A; Wolf David E

CORPORATE SOURCE: Sensor Technologies, LLC, Shrewsbury, Massachusetts 01545,

USA.

CONTRACT NUMBER: NS-07366 (NINDS)

NS28760 (NINDS)

SOURCE: Methods in cell biology, (2003) Vol. 72, pp. 465-98.

Journal code: 0373334. ISSN: 0091-679X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200403

Entered STN: 15 Jan 2004 ENTRY DATE:

> Last Updated on STN: 6 Mar 2004 Entered Medline: 5 Mar 2004

AB FCS is an important technique for biophysicists, biochemists, and cell biologists. FCS represents an example of how one can make use of the microscope and electronics to extract information beyond the resolution limit of classical optics. It can be used to study single-molecules both in solution and in living cells and can be used to monitor a wide variety of macromolecular interactions. When used as an in vitro technique, FCS measurements are easy to conduct and can be made on simplified instrumentation. When used in vivo on living cells, many additional factors must be considered when evaluating experimental data. Despite these concerns, FCS represents a new approach that has broad applicability for the determination of molecular stoichiometry both in vivo and in vitro for a variety of membrane and soluble receptor systems.

ANSWER 2 OF 7 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN L7 2000:191522 BIOSIS

ACCESSION NUMBER: DOCUMENT NUMBER:

SOURCE:

PREV200000191522

TITLE: Global analysis of FCS data: Diffusion of DiIC16 and gp75

> in the plasma membrane of A875 human melanoma cells. Bulseco, Dylan A.; Thompson, Christine A.; Browne,

AUTHOR(S): Elizabeth S.; Quinby, Peter R.; Wolf, David E.

Biophysical Journal, (Jan., 2000) Vol. 78, No. 1 Part 2,

pp. 441A. print.

Meeting Info.: 44th Annual Meeting of the Biophysical

Society. New Orleans, Louisiana, USA. February 12-16, 2000.

CODEN: BIOJAU. ISSN: 0006-3495.

DOCUMENT TYPE: Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)

Conference; (Meeting Poster)

LANGUAGE:

English

ENTRY DATE:

Entered STN: 17 May 2000

Last Updated on STN: 4 Jan 2002

ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN 1.7

ACCESSION NUMBER: 1998:302684 BIOSIS DOCUMENT NUMBER: PREV199800302684

TITLE: Intranuclear diffusion and hybridization state of

> oligonucleotides measured by fluorescence correlation spectroscopy in living cells.

Politz, Joan C. [Reprint author]; Browne, Elizabeth S.; AUTHOR(S):

Wolf, David E.; Pederson, Thoru

Dep. Biochem. Mol. Biol., Univ. Mass. Med. Cent., Worcester CORPORATE SOURCE:

Found. Campus, 222 Maple Ave., Shrewsbury, MA 01545, USA Proceedings of the National Academy of Sciences of the

SOURCE: United States of America, (May 26, 1998) Vol. 95, No. 11,

pp. 6043-6048. print.

CODEN: PNASA6. ISSN: 0027-8424.

DOCUMENT TYPE: Article English LANGUAGE:

ENTRY DATE: Entered STN: 15 Jul 1998

Last Updated on STN: 15 Jul 1998

Fluorescein-labeled oligodeoxynucleotides (oligos) were introduced into AB cultured rat myoblasts, and their molecular movements inside the nucleus were studied by fluorescence correlation spectroscopy (FCS) and fluorescence recovery after photobleaching (FRAP). FCS revealed that a large fraction of both intranuclear oligo(dT) (43%) and oligo(dA) (77%) moves rapidly with a diffusion coefficient of 4 X 10-7 cm2/s. Interestingly, this rate of intranuclear oligo movement is similar to their diffusion rates measured in aqueous solution. In addition, we detected a large fraction (45%) of the intranuclear oligo(dT), but not oligo(dA), diffusing at slower rates (ltoreq1  $\times$  10-7 cm2/s). The amount of this slower-moving oligo(dt) was greatly reduced if the oligo(dt) was prehybridized in solution with (unlabeled) oligo(dA) prior to introduction to cells, presumably because the oligo(dt) was then unavailable for subsequent hybridization to endogenous poly(A) RNA. The FCS-measured diffusion rate for much of the slower oligo(dt) population approximated the diffusion rate in aqueous solution of oligo(dt) hybridized to a large polyadenylated RNA (1.0 X 10-7 cm2/s). Moreover, this intranuclear movement rate falls within the range of calculated diffusion rates for an average-sized heterogeneous nuclear ribonucleoprotein particle in aqueous solution. A subfraction of oligo(dt) (15%) moved over 10-fold more slowly, suggesting it was bound to very large macromolecular complexes. Average diffusion coefficients obtained from FRAP experiments were in agreement with the FCS data. These results demonstrate that oligos can move about within the nucleus at rates comparable to those in aqueous solution and further suggest that this is true for large ribonucleoprotein complexes as well.

ANSWER 4 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN T.7

ACCESSION NUMBER: 2004:121051 CAPLUS

DOCUMENT NUMBER: 140:171955

TITLE: Fluorescence correlation spectroscopy instrument

INVENTOR(S): Bulseco, Dylan A.; Wolf, David E. PATENT ASSIGNEE(S): Sensor Technologies LLC, USA

SOURCE: PCT Int. Appl., 47 pp.

CODEN: PIXXD2

DOCUMENT TYPE: LANGUAGE:

Patent English

FAMILY ACC. NUM. COUNT:

PATENT INFORMATION:

PATEN	PATENT NO.				KIND DATE			APPLICATION NO.					DATE			
WO 20	040136	20				2004	0212							2	0030	801
W	: AE,	AG,	AL,	AM,	AT,	AU,	ΑZ,	BA,	BB,	BG,	BR,	BY,	BZ,	CA,	CH,	CN,
	co,	CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD,	GE,	GH,
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	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,	MZ,	NO,	NZ,	OM,	PH,
	PL,	PT,	RO,	RU,	SD,	SE,	SG,	SK,	SL,	TJ,	TM,	TN,	TR,	TT,	TZ,	UA,
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R'	W: GH,	GM,	KE,	LS,	MW,	MZ,	SD,	SL,	SZ,	TZ,	ŪĠ,	ZM,	ZW,	AM,	AZ,	BY,
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	BF,	ВJ,	CF,	CG,	CI,	CM,	GA,	GN,	GQ,	GW,	ML,	MR,	NE,	SN,	TD,	TG
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US 20	040807	50		<b>A</b> 1		2004	0429	1	US 2	003-	6333	85		2	0030	801
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JP 20	055349	44		Т2		2005	1117		JP 2	005-	5060	96		2	0030	801
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								1	US 2	003-	4613	94P	1	P 2	0030	408
								1	WO 2	003-1	US24:	369	1	W 2	0030	801

AB A portable fluorescence correlation spectroscopy instrument is described comprising an excitation source, at least one of a light focusing element positioned to receive light emitted by the excitation source, a detector for detecting light, the detector positioned to receive light emitted by a sample excited by the excitation source, and a correlator coupled to the detector, the correlator for processing data received at the detector and providing data including autocorrelation data, crosscorrelation data, or a combination thereof.

ANSWER 5 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:121045 CAPLUS

DOCUMENT NUMBER: 140:177861

TITLE: Method of measuring molecular interactions

Wolf, David E.; Bulseco, Dylan A. INVENTOR(S): Sensor Technologies LLC, USA PATENT ASSIGNEE(S):

PCT Int. Appl., 76 pp. SOURCE:

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATEN	NO.			KIN	D :	DATE		i	APPL	ICAT	ION 1	NO.		D	ATE	
WO 200		- •		A2 A3		2004 2004		1	WO 2	003-	US24	780		2	0030	801
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RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,
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PRIORITY APPLN. INFO.:
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A method of assaying for the equilibrium interaction of a probe and an unknown AΒ target, is described entailing exciting a sample at with radiation, the sample comprising at least a portion of the members of a library, at least one probe, and at least one fluorescent tag; measuring the fluorescence from a subvolume of the sample; and analyzing the fluctuations of the fluorescence. The method may apply for assaying for a pathogen (e.g., bacterium, virus) in a sample. method may apply for assaying for the presence of a toxin (e.g., ricin) in a sample. A kit comprising a first probe comprising ricin, a fluorescent tag attached to the ricin; and a second probe bound to the first probe, the second probe being adapted to bind ricin is also described. A method of determining a true crosscorrelation function of a sample is also described entailing obtaining a first measured correlation function of the sample from a first detector of a fluorescence correlation spectroscopy instrument; obtaining a second measured correlation function of the sample from a second detector of the instrument; obtaining a measured crosscorrelation function between the first detector and the second detector of the instrument; determining a true crosscorrelation function.

An article of manufacture comprising a computer readable medium having stored therein a computer program for determining a true correlation function of a sample, wherein the computer program comprises a first code segment for obtaining a measured correlation function of the sample; and a second code segment for applying a correction algorithm to the measured correlation function is also described. A fluorescence correlation spectrometer for determining a true correlation function of a sample is also described comprising an excitation source; a first detector and a second detector for measuring fluorescence of the sample; a memory device for storing information related to the sample; and a processor programmed with instruction to obtain a measured correlation function of the sample from a fluorescence correlation spectroscopy instrument and apply a correction algorithm to the measured correlation function.

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L7 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN
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ACCESSION NUMBER: 1998:806814 CAPLUS

DOCUMENT NUMBER: 130:49505

TITLE: Method for determining rate of movement of a molecule

in a living cell

INVENTOR(S): Politz, Joan C.; Wolf, David E.; Browne,

Elizabeth S.; Pederson, Thoru University of Massachusetts, USA

PATENT ASSIGNEE(S): University of Massachu SOURCE: PCT Int. Appl., 44 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

## PATENT INFORMATION:

W: JP

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT SE

PRIORITY APPLN. INFO.:

US 1997-49852P P 19970605

AB A method for determining the rate of movement of a mol. inside a living cell by fluorescence correlation spectroscopy (FCS) is disclosed. Also disclosed is a method for detecting hybridization of a nucleic acid inside a living cell. The figure is a graph showing autocorrelation curves and best fit curves for fluorescently-labeled oligonucleotides in nuclei of L6 rat myoblasts.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1998:336307 CAPLUS

DOCUMENT NUMBER: 129:93313

TITLE: Intranuclear diffusion and hybridization state of

oligonucleotides measured by fluorescence correlation spectroscopy in living cells

AUTHOR(S): Politz, Joan C.; Browne, Elizabeth S.; Wolf,

David E.; Pederson, Thoru

CORPORATE SOURCE: Worcester Foundation Biomedical Res. Dep. Biochem.

Molecular Biol., Univ. Massachusetts Medical Cent.,

Shrewsbury, MA, 01545, USA

SOURCE: Proceedings of the National Academy of Sciences of the

United States of America (1998), 95(11), 6043-6048

CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal LANGUAGE: English

AB Fluorescein-labeled oligodeoxynucleotides (oligos) were introduced into cultured rat myoblasts, and their mol. movements inside the nucleus were studied by fluorescence correlation spectroscopy (FCS) and fluorescence recovery after photobleaching (FRAP). FCS revealed that a large fraction of both intranuclear oligo(dT) (43%) and oligo(dA) (77%) moves rapidly with a diffusion coefficient of 4 + 10-7 cm2/s. Interestingly, this rate of intranuclear oligo movement is similar to their diffusion rates measured in aqueous solution. In addition, we detected a

large

fraction (45%) of the intranuclear oligo(dT), but not oligo(dA), diffusing at slower rates (≤1 + 10-7 cm2/s). The amount of this slower-moving oligo(dT) was greatly reduced if the oligo(dT) was prehybridized in solution with (unlabeled) oligo(dA) prior to introduction to cells, presumably because the oligo(dT) was then unavailable for subsequent hybridization to endogenous poly(A) RNA. The FCS-measured diffusion rate for much of the slower oligo(dT) population approximated the diffusion rate in aqueous solution of oligo(dT) hybridized to a large polyadenylated RNA (1.0 + 10-7 cm2/s). Moreover, this intranuclear movement rate falls within the range of calculated diffusion rates for an average-sized heterogeneous nuclear ribonucleoprotein particle in aqueous solution A

subfraction of oligo(dT) (15%) moved over 10-fold more slowly, suggesting it was bound to very large macromol. complexes. Average diffusion coeffs. obtained from FRAP expts. were in agreement with the FCS data. These results demonstrate that oligos can move about within the nucleus at rates comparable to those in aqueous solution and further suggest that this is true

for

large ribonucleoprotein complexes as well.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ibib abs 18 1-21

L8 ANSWER 1 OF 21 MEDLINE on STN ACCESSION NUMBER: 97075073 MEDLINE

DOCUMENT NUMBER: PubMed ID: 8917500

TITLE: Fluorescence correlation analysis of

probe diffusion simplifies quantitative pathogen

detection by PCR.

AUTHOR: Walter N G; Schwille P; Eigen M

CORPORATE SOURCE: Department of Biochemical Kinetics, Max Planck Institute

for Biophysical Chemistry, Gottingen, Germany.

SOURCE: Proceedings of the National Academy of Sciences of the

United States of America, (1996 Nov 12) Vol. 93, No. 23,

pp. 12805-10.

Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199612

ENTRY DATE: Entered STN: 28 Jan 1997

Last Updated on STN: 6 Feb 1998 Entered Medline: 30 Dec 1996

A sensitive, labor-saving, and easily automatable nonradioactive procedure AB named APEX-FCS (amplified probe extension detected by fluorescence correlation spectroscopy) has been established to detect specific in vitro amplification of pathogen genomic sequences. As an example, Mycobacterium tuberculosis genomic DNA was subjected to PCR amplification with the Stoffel fragment of Thermus aquaticus DNA polymerase in the presence of nanomolar concentrations of a rhodamine-labeled probe (third primer), binding to the target in between the micromolar amplification primers. The probe becomes extended only when specific amplification occurs. Its low concentration avoids false-positives due to unspecific hybridization under PCR conditions. With increasing portion of extended probe molecules, the probe's average translational diffusion properties gradually change over the course of the reaction, reflecting amplification kinetics. Following PCR, this change from a stage of high to a stage of low mobility can directly be monitored during a 30-s measurement using a fluorescence correlation spectroscopy device. Quantitation down to 10 target molecules in a background of 2.5 micrograms unspecific DNA without post-PCR probe manipulations could be achieved with different primer/ probe combinations. The assay holds the promise to concurrently perform amplification, probe hybridization, and specific detection without opening the reaction chamber, if sealable foils are used.

L8 ANSWER 2 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:250891 BIOSIS DOCUMENT NUMBER: PREV200600243875

TITLE: Detection of prion particles in samples of BSE and scrapie

by fluorescence correlation

spectroscopy without proteinase K digestion.

AUTHOR(S): Birkmann, Eva; Schaefer, Oliver; Weinmann, Nicole;

Dumpitak, Christian; Beekes, Michael; Jackman, Roy; Thorne,

Leigh; Riesner, Detlev [Reprint Author]

CORPORATE SOURCE: Univ Dusseldorf, Inst Biol Phys, Univ Str 1, D-40225

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SOURCE: Biological Chemistry, (JAN 2006) Vol. 387, No. 1, pp.

95-102.

ISSN: 1431-6730.

DOCUMENT TYPE: LANGUAGE:

Article English

ENTRY DATE:

Entered STN: 26 Apr 2006

Last Updated on STN: 26 Apr 2006

A characteristic feature of prion diseases such as bovine spongiform encephalopathy (BSE) is the accumulation of a pathological isoform of the host-encoded prion protein, PrP. In contrast to its cellular isoform PrPC, the pathological isoform PrPSc forms insoluble aggregates. All commercial BSE tests currently used for routine testing are based on the proteinase K (PK) resistance of PrP, but not all pathological PrP is PK-resistant. In the present study, single prion particles were counted by fluorescence correlation spectroscopy (FCS). The property of PK resistance is not required, i.e., both the PK-resistant and the PK-sensitive parts of the prion particles are detectable. PrP aggregates were prepared from the brains of BSE-infected cattle, as well as from scrapie-infected hamsters, by the NaPTA precipitation method without PK digestion. They were labeled using two different PrP-specific antibodies for FCS measurements in the dual-color mode (2D-FIDA). Within the limited number of samples tested, BSE-infected cattle and scrapie-infected hamsters in the clinical stage of the disease could be distinguished with 100% specificity from a control group. Thus, a diagnostic tool for BSE detection with complete avoidance of PK treatment is presented, which should have particular advantages for testing animals in the preclinical stage.

L8 ANSWER 3 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: DOCUMENT NUMBER:

2006:127126 BIOSIS PREV200600114592

TITLE:

Disassembly of structurally modified viral nanoparticles:

characterization by fluorescence

correlation spectroscopy.

AUTHOR(S):

Toivola, Jouni; Gilbert, Leona; Michel, Patrik; White, Daniel; Vuento, Matti; Oker-Blom, Christian [Reprint

Author]

CORPORATE SOURCE:

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SOURCE:

Comptes Rendus Biologies, (DEC 2005) Vol. 328, No. 12, pp.

1052-1056.

ISSN: 1631-0691.

DOCUMENT TYPE: LANGUAGE:

Article English

ENTRY DATE:

Entered STN: 15 Feb 2006

Last Updated on STN: 15 Feb 2006

AB Analysis of the breakdown products of engineered viral particles can give useful information on the particle structure. We used various methods to breakdown both a recombinant enveloped virus and virus-like particles (VLPs) from two non-enveloped viruses and analysed the resulting subunits by fluorescence correlation spectroscopy (FCS).

Analysis of the enveloped baculovirus Autographa californica multicapsid

Analysis of the enveloped baculovirus, Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV), displaying the green fluorescent protein (GFP) fused to its envelope protein gp64 was performed in the presence and absence of 5 mM SDS and 25 mM DTT. Without treatment, the viral particle showed a diffusion time of 3.3 ms. In the presence of SDS, fluorescent subunits with diffusion times of 0.2 ms were observed. Additional treatment with DTT caused a drop in the diffusion time to 0.1 ms. Changes in the amplitude of the autocorrelation function suggested a 3-fold increase in fluorescent particle number when viral particles were treated

with SDS, and a further 1.5-fold increase with additional treatment with Thus, the data showed that an average of 4.5 molecules of qp64-GFP was incorporated in the membrane of the modified baculovirus. Further, this suggests that each fluorescent gp64 trimer carries on average 1.5 fluorescent units. Similar experiments were carried out with two non-enveloped fluorescent virus-like particles (fVLPs) that displayed enhanced green fluorescent protein (EGFP). These, fVLPs of canine and human B 19 parvoviruses were treated with 6 M urea and 5 mM SDS, respectively. Correspondingly, the original hydrodynamic radii of 17 and 14 nm were reduced to 9 and 5 nm after treatment. Here, the change in the amplitude of the autocorrelation curve suggested a 10-fold increase in particle number when viral particles of CPV were treated with 6 M urea at 50 degrees C for 10 min. For EGFP-13 19, there was a decrease in the amplitude, accompanied by a 9-fold increase in the number of fluorescent units with SDS treatment. The results showed that approximately 10 and 9 fluorescent units were associated with the corresponding CPV and B 19 VLPs. In summary, we were able to estimate the number of fluorescent subunits in a baculovirus containing a GFP-fusion with its qp64 envelope protein and in two different parvo-VLPs containing EGFP-fused with their VP2 capsid proteins.

L8 ANSWER 4 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:86447 BIOSIS DOCUMENT NUMBER: PREV200600080673

TITLE: Membrane perturbation effects of peptides derived from the

N-termini of unprocessed prion proteins.

AUTHOR(S): Mapzoub, Mazin; Oglecka, Kamila; Pramanik, Aladdin;

Eriksson, L. E. Goran; Graslund, Astrid [Reprint Author]

CORPORATE SOURCE: Stockholm Univ, Arrhenius Lab, Dept Biochem and Biophys,

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SOURCE: Biochimica et Biophysica Acta, (OCT 15 2005) Vol. 1716, No.

2, pp. 126-136. ISSN: 0005-2736.

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 25 Jan 2006

Last Updated on STN: 25 Jan 2006

Peptides derived from the unprocessed N-termini of mouse and bovine prion proteins (mPrPp and bPrPp, respectively), comprising hydrophobic signal sequences followed by charged domains (KKRPKP), function as cell-penetrating peptides (CPPs) with live cells, concomitantly causing toxicity. Using steady-state fluorescence techniques, including calcein leakage and polarization of a membrane probe (diphenylhexatriene, DPH), as well as circular dichroism, we studied the membrane interactions of the peptides with large unilamellar phospholipid vesicles (LUVs), generally with a 30% negative surface charged density, comparing the effects with those of the CPP penetratin (pAntp) and the pore-forming peptide melittin. The prion peptides caused significant calcein leakage from LUVs concomitant with increased membrane ordering. Fluorescence correlation spectroscopy (FCS) studies of either rhodamine-entrapping (REVs) or rhodamine-labeled (RLVs) vesicles, showed that addition of the prion peptides resulted in significant release of rhodamine from the REVs without affecting the overall integrity of the RLVs. The membrane leakage effects due to the peptides had the following order of potency: melittin > mPrPp > bPrPp > pAntp. The membrane perturbation effects of the N-terminal prion peptides suggest that they form transient pores (similar to melittin) causing toxicity in parallel with their cellular trafficking. (c) 2005 Elsevier B.V. All rights reserved.

ACCESSION NUMBER: 2005:303857 BIOSIS DOCUMENT NUMBER: PREV200510086544

TITLE: Molecular and structural characterization of fluorescent

human parvovirus B19 virus-like particles.

AUTHOR(S): Gilbert, Leona; Toivola, Jouni; White, Daniel; Ihalainen,

Teemu; Smith, Wesley; Lindholm, Laura; Vuento, Matti;

Oker-Blom, Christian [Reprint Author]

CORPORATE SOURCE: Univ Jyvaskyla, Dept Biol and Environm Sci, POB 35,

FIN-40014 Jyvaskyla, Finland christian.oker-blom@jyu.fi

SOURCE: Biochemical and Biophysical Research Communications, (JUN 3

2005) Vol. 331, No. 2, pp. 527-535.

CODEN: BBRCA9. ISSN: 0006-291X.

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 15 Aug 2005

Last Updated on STN: 15 Aug 2005

Although sharing a T = 1 icosahedral symmetry with other members of the Parvoviridae family, it has been suggested that the fivefold channel of the human parvovirus B19 VP2 capsids is closed at its outside end. To investigate the possibility of placing a relatively large protein moiety at this site of 1319, fluorescent virus-like particles (fVLPs) of B19 were developed. The enhanced green fluorescent protein (EGFP) was inserted at the N-terminus of the structural protein VP2 and assembly of fVLPs from this fusion protein was obtained. Electron microscopy revealed that these fluorescent protein complexes were very similar in size when compared to wild-type B19 virus. Further, fluorescence correlation spectroscopy showed that an average of nine EGFP domains were associated with these virus-like structures. Atomic force microscopy and immunoprecipitation studies showed that EGFP was displayed on the surface of these fVLPs. Confocal imaging indicated that these chimeric complexes were targeted to late endosomes when expressed in insect cells. The FVLPs were able to efficiently enter cancer cells and traffic to the nucleus via the microtubulus network. Finally, immunoglobulins present in human parvovirus B19 acute and past-immunity serum samples were able to detect antigenic epitopes present in these fVLPs. In summary, we have developed fluorescent virus-like nanoparticles displaying a large heterologous entity that should be of help to elucidate the mechanisms of infection and pathogenesis of human parvovirus B19. In addition, these B 19 nanoparticles serve as a model in the development of targetable vehicles designed for delivery of biomolecules. (c) 2005 Elsevier Inc. All rights reserved.

L8 ANSWER 6 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:235886 BIOSIS DOCUMENT NUMBER: PREV200510020951

TITLE: Measuring protein-protein interactions inside living cells

using single color fluorescence

correlation spectroscopy. Application to human

immunodeficiency virus type I integrase and LEDGF/p75.

AUTHOR(S): Maertens, Goedele; Vercammen, Jo; Debyser, Zeger;

Engelborghs, Yves [Reprint Author]

CORPORATE SOURCE: Katholieke Univ Leuven, Lab Biomol Dynam, Celestijnenlaan

200D, B-3001 Louvain, Belgium

Yves.Engelborghs@fys.kuleuven.ac.be

SOURCE: FASEB Journal, (MAR 2005) Vol. 19, No. 3.

CODEN: FAJOEC. ISSN: 0892-6638.

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 23 Jun 2005

Last Updated on STN: 23 Jun 2005

AB Recently we described the interaction of human immunodeficiency virus type

epithelium-derived growth factor/transcription coactivator p75 (LEDGF/p75). We now present the study of the diffusion behavior of the three independent domains of IN and LEDGF/p75 using fluorescence correlation microscopy (FCM). We show that diffusion in the cell of the different enhanced green fluorescent protein (EGFP) fusion proteins is described by two components with different fractions and that the average parameters in the nucleus are comparable with those in the cytoplasm. In addition, we demonstrate that specific interaction between EGFP-fused HIV-1 IN and LEDGF/p75 results in a shift in diffusion coefficient (D). The opposite shift was observed in an IN-deletion mutant that does not exhibit LEDGF/p75 binding or in a LEDGF/p75 knock-down experiment using siRNA. We thus demonstrate that protein-protein interactions can be studied in living cells, using single-color FCM (scFCM).

L8 ANSWER 7 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:114490 BIOSIS DOCUMENT NUMBER: PREV200500111906

TITLE: Observation of a fluorescently labelled virus on its

infectious entry pathway into the cell using

Fluorescence Correlation Spectroscopy.

AUTHOR(S): Bernacchi, Serena; Mueller, Gabriele; Langowski, Joerg;

Waldeck, Waldemar

SOURCE: Biochemical Society Transactions, (August 2004) Vol. 32,

No. Part 4, pp. 121A. print.

Meeting Info.: BioScience2004: From Molecules to Organisms.

Glasgow, UK. July 18-22, 2004. The Biochemical Society.

CODEN: BCSTB5. ISSN: 0300-5127.

DOCUMENT TYPE: Conference; (Meeting)

Conference; (Meeting Poster)

LANGUAGE: English

ENTRY DATE: Entered STN: 23 Mar 2005

Last Updated on STN: 23 Mar 2005

L8 ANSWER 8 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:461167 BIOSIS DOCUMENT NUMBER: PREV200400463059

TITLE: HIV-1 nucleocapsid protein binds to the viral DNA

initiation sequences and chaperones their kissing

interactions.

AUTHOR(S): Egele, Caroline; Schaub, Emmanuel; Ramalanjaona, Nick;

Piemont, Etienne; Ficheux, Damien; Roques, Bernard; Darlix,

Jean-Luc; Mely, Yves [Reprint Author]

CORPORATE SOURCE: Fac PharmCNRSUMR 7034, Lab Pharmacol and Phys Chim Interact

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F-67401, Illkirch Graffenstaden, France

mely@pharma.u-strasbg.fr

SOURCE: Journal of Molecular Biology, (September 10 2004) Vol. 342,

No. 2, pp. 453-466. print. ISSN: 0022-2836 (ISSN print).

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 1 Dec 2004

Last Updated on STN: 1 Dec 2004

AB The chaperone properties of the human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein (NC) are required for the two obligatory strand transfer reactions occurring during viral DNA synthesis. The second strand transfer relies on the destabilization and the subsequent annealing of the primer binding site sequences (PBS) at the 3' end of the (-) and (+) DNA strands. To characterize the binding and chaperone properties of NC on the (-) PBS and (+) PBS sequences, we monitored by

steady-state and time-resolved fluorescence spectroscopy as well as by fluorescence correlation spectroscopy the interaction of NC with wild type and mutant oligonucleotides corresponding to the (-)PBS and (+) PBS hairpins. NC was found to bind with high affinity to the loop, the stem and the single-stranded protruding sequence of both PBS sequences. NC induces only a limited destabilization of the secondary structure of both sequences, activating the transient melting of the stem only during its "breathing" period. This probably results from the high stability of the PBS due to the four G-C pairs in the stem. In contrast, NC directs the formation of "kissing" homodimers efficiently for both (-) PBS and (+) PBS sequences. Salt-induced dimerization and mutations in the (-) PBS sequence suggest that these homodimers may be stabilized by two intermolecular G-C Watson-Crick base-pairs between the partly self-complementary loops. The propensity of NC to promote the dimerization of partly complementary sequences may favor secondary contacts between viral sequences and thus, recombination and viral diversity. Copyright 2004 Elsevier Ltd. All rights reserved.

L8 ANSWER 9 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:389740 BIOSIS DOCUMENT NUMBER: PREV200400392380

TITLE: Effect of metal ions on de novo aggregation of full-length

prion protein.

AUTHOR(S): Giese, Armin [Reprint Author]; Levin, Johannes; Bertsch,

Uwe; Kretzschmar, Hans

CORPORATE SOURCE: Zentrum Neuropathol and Prionforschung, Univ Munich,

Munich, Germany

Armin.Giese@med.uni-muenchen.de

SOURCE: Biochemical and Biophysical Research Communications,

(August 6 2004) Vol. 320, No. 4, pp. 1240-1246. print.

CODEN: BBRCA9. ISSN: 0006-291X.

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 6 Oct 2004

Last Updated on STN: 6 Oct 2004

AB It is well established that the prion protein (PrP) contains metal ion binding sites with specificity for copper. Changes in copper levels have been suggested to influence incubation time in experimental prion disease. Therefore, we studied the effect of heavy metal ions (Cu2+, Mn2+, Ni2+, Co2+, and Zn2+) in vitro in a model system that utilizes changes in the concentration of SDS to induce structural conversion and aggregation of recombinant PrP. To quantify and characterize PrP aggregates, we used fluoreseently labelled PrP and cross-correlation analysis as well as scanning for intensely fluorescent targets in a confocal single molecule detection system. We found a specific strong pro-aggregatory effect of Mn2+ at low micromolar concentrations that could be blocked by nanomolar concentration of Cu2+. These findings suggest that metal ions such as copper and manganese may also affect PrP conversion in vivo. Copyright 2004 Elsevier Inc. All rights reserved.

L8 ANSWER 10 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:155646 BIOSIS DOCUMENT NUMBER: PREV200400156914

TITLE: Monitoring human parvovirus B19 virus-like particles and

antibody complexes in solution by fluorescence

correlation spectroscopy.

AUTHOR(S): Toivola, Jouni; Michel, Patrik O.; Gilbert, Leona;

Lahtinen, Tomi; Marjomaki, Varpu; Hedman, Klaus; Vuento,

Matti; Oker-Blom, Christian [Reprint Author]

CORPORATE SOURCE: Department of Biological and Environmental Science,

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SOURCE: Biological Chemistry, (January 2004) Vol. 385, No. 1, pp.

87-93. print. ISSN: 1431-6730.

DOCUMENT TYPE: Article

LANGUAGE: English
ENTRY DATE: Entered STN: 17 Mar 2004

Last Updated on STN: 17 Mar 2004

Fluorescence correlation spectroscopy (FCS) was used AB in monitoring human parvovirus B19 virus-like particle (VLP) antibody complexes from acute phase and past-immunity serum samples. The Oregon Green 488-labeled VLPs gave an average diffusion coefficient of 1.7X10-7 cm2s-1 with an apparent hydrodynamic radius of 14 nm. After incubation of the fluorescent VLPs with an acute phase serum sample, the mobility information obtained from the fluorescence intensity fluctuation by autocorrelation analysis showed an average diffusion coefficient of 1.5X10-8 cm2s-1, corresponding to an average radius of 157 nm. In contrast, incubation of the fluorescent VLPs with a past-immunity serum sample gave an average diffusion coefficient of 3.5X10-8 cm2s-1 and a radius of 69 nm. A control serum devoid of B19 antibodies caused a change in the diffusion coefficient from 1.7X10-7 to 1.6X10-7 cm2s-1, which is much smaller than that observed with acute phase or past-immunity sera. Thus, VLP-antibody complexes with different diffusion coefficients could be identified for the acute phase and past-immunity sera. FCS measurement of VLP-immune complexes could be useful in distinguishing between antibodies present in acute phase or

L8 ANSWER 11 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on

past-immunity sera as well as in titration of the VLPs.

STN

ACCESSION NUMBER: 2004:146893 BIOSIS DOCUMENT NUMBER: PREV200400151029

TITLE: Assembly of fluorescent chimeric virus-like particles of

canine parvovirus in insect cells.

AUTHOR(S): Gilbert, L.; Toivola, J.; Lehtomaki, E.; Donaldson, L.;

Kapyla, P.; Vuento, M.; Oker-Blom, C. [Reprint Author]

CORPORATE SOURCE: Department of Biological and Environmental Science,

University of Jyvaskyla, P.O. Box 35, Jyvaskyla, FIN-40014,

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SOURCE: Biochemical and Biophysical Research Communications,

(January 23 2004) Vol. 313, No. 4, pp. 878-887. print.

CODEN: BBRCA9. ISSN: 0006-291X.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 17 Mar 2004

Last Updated on STN: 17 Mar 2004

AB Canine parvovirus (CPV) is a small non-enveloped ssDNA virus composed of the viral proteins VP1, VP2, and VP3 with a T=1 icosahedral symmetry. VP2 is nested in VP1 and the two proteins are produced by differential splicing of a primary transcript of the right ORF of the viral genome. The VP2 protein can be further proteolytically cleaved to form VP3. Previous studies have shown that VP1 and VP3 are unnecessary for capsid formation and consequently, that VP2 alone is sufficient for assembly. We have hypothesized that insertion of the enhanced green fluorescent protein (EGFP) at the N-terminus of VP2 could be carried out without altering assembly. To investigate the possibility to develop fluorescent virus-like particles (fVLPs) from such chimeric VP2 proteins, the corresponding fusion construct was abundantly expressed in insect cells. Confocal imaging indicated that the EGFP-VP2 fusion product was assembled to fluorescent capsid-like complexes. In addition, electron micrographs

of purified EGFP-VP2 complexes showed that they displayed a very similar size and appearance when compared to VP2 VLPs. Further, immunolabelling of purified EGFP-VP2 VLPs showed the presence of EGFP within the structure. Fluorescence correlation spectroscopy (FCS) studies confirmed that fVLPs were very similar in size when compared to authentic CPV. Finally, feeding of mammalian cells susceptible to CPV infection with these fVLPs indicated that entry and intracellular trafficking could be observed. In summary, we have developed fluorescent virus-like nanoparticles carrying a heterologous entity that can be utilized as a visualization tool to elucidate events related to a canine parvovirus infection.

L8 ANSWER 12 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on

STN

ACCESSION NUMBER: 2003:509999 BIOSIS DOCUMENT NUMBER: PREV200300496950

TITLE: Direct measurement of Gag-Gag interaction during retrovirus

assembly with FRET and fluorescence

correlation spectroscopy.

AUTHOR(S): Larson, Daniel R.; Ma, Yu May; Vogt, Volker M.; Webb, Watt

W. [Reprint Author]

CORPORATE SOURCE: Cornell University School of Applied and Engineering

Physics, 212 Clark Hall, Ithaca, NY, 14853, USA

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SOURCE: Journal of Cell Biology, (September 29 2003) Vol. 162, No.

7, pp. 1233-1244. print.

CODEN: JCLBA3. ISSN: 0021-9525.

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 29 Oct 2003

Last Updated on STN: 29 Oct 2003

AB During retrovirus assembly, the polyprotein Gag directs protein multimerization, membrane binding, and RNA packaging. It is unknown whether assembly initiates through Gag-Gag interactions in the cytosol or at the plasma membrane. We used two fluorescence techniques-two-photon fluorescence resonance energy transfer and fluorescence correlation spectroscopy-to examine Rous sarcoma virus Gag-Gag and -membrane interactions in living cells. Both techniques provide strong evidence for interactions between Gag proteins in the cytoplasm. Fluorescence correlation spectroscopy measurements of mobility suggest that Gag is present in large cytosolic complexes, but these complexes are not entirely composed of Gag. Deletion of the nucleocapsid domain abolishes Gag interactions and membrane targeting. Deletion of the membrane-binding domain leads to enhanced cytosolic interactions. These results indicate that Gag-Gag interactions occur in the cytosol, are mediated by nucleocapsid domain, and are necessary for membrane targeting and budding. These methods also have general applicability to in vivo studies of protein-protein and -membrane interactions involved in the formation of complex macromolecular structures.

L8 ANSWER 13 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on

STN

ACCESSION NUMBER: 2003:161076 BIOSIS DOCUMENT NUMBER: PREV200300161076

TITLE: Destabilization of the HIV-1 complementary sequence of TAR

by the nucleocapsid protein through activation of

conformational fluctuations.

AUTHOR(S): Azoulay, Joel; Clamme, Jean-Pierre; Darlix, Jean-Luc;

Roques, Bernard P.; Mely, Yves [Reprint Author]

CORPORATE SOURCE: Laboratoire de Pharmacologie et Physico-Chimie des

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SOURCE: Journal of Molecular Biology, (21 February 2003) Vol. 326,

No. 3, pp. 691-700. print. ISSN: 0022-2836 (ISSN print).

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 26 Mar 2003

Last Updated on STN: 26 Mar 2003

AB The nucleocapsid protein NCp7 of HIV-1 possesses nucleic acid chaperone properties that are critical for the two obligatory strand transfer reactions required for the synthesis of a complete proviral DNA by reverse transcriptase. The first DNA strand transfer relies on the destabilization by NCp7 of double-stranded segments of the transactivation response region (TAR) sequence at the 3' end of the genomic RNA and the complementary sequence cTAR at the 3' terminus of minus strong-stop DNA, the early product of reverse transcription. In order to determine the dynamics of NCp7-mediated nucleic acid destabilization, we investigated by time-resolved fluorescence spectroscopy and two photon fluorescence correlation spectroscopy, the interaction of a doubly labeled cTAR sequence with NC(12-55) containing NCp7 CCHC zinc fingers and flanking basic amino acid residues. From the chemical rates and the activation energy associated with the conformational fluctuations observed in the absence of NC, it is concluded that such fluctuations are associated with the opening and closing of the double-stranded terminal segments of cTAR. The destabilizing activity of NC(12-55) occurs mainly through a major increase of the opening rate constant of cTAR. Moreover, NC appears to augment the number of pathways between the open and closed states of cTAR, suggesting that it initiates melting of base-pairs at different locations within the terminal segments of cTAR. This activity of NC on the dynamics of cTAR secondary structure is thought to be critical for the formation of the cTAR-TAR complex, which is essential for the specificity and extent of proviral DNA synthesis by reverse transcriptase.

L8 ANSWER 14 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on

STN

ACCESSION NUMBER: 2002:371152 BIOSIS DOCUMENT NUMBER: PREV200200371152

TITLE: Fluorescence correlation spectroscopy

for in vivo monitoring of resistance mechanisms.

AUTHOR(S): Schots, A. [Reprint author]; Dees, R. [Reprint author];

Goverse, A.; Borst, J. W. [Reprint author]; Bakker, J.;

Visser, A. J. W. G.

CORPORATE SOURCE: Lab. of Molecular Recognition and Antibody Technology,

Wageningen University, Wageningen, Netherlands

SOURCE: Journal of Nematology, (December, 2001) Vol. 33, No. 4, pp.

276. print.

Meeting Info.: 40th Annual Meeting of the Society of Nematologists. Salt Lake City, Utah, USA. August 25-29,

2001.

CODEN: JONEB5. ISSN: 0022-300X.

DOCUMENT TYPE: Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 3 Jul 2002

Last Updated on STN: 3 Jul 2002

L8 ANSWER 15 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on

STN

ACCESSION NUMBER: 2002:360914 BIOSIS

DOCUMENT NUMBER: PREV200200360914

TITLE: Molecular diagnostics of transmissible spongiform

encephalopathies.

AUTHOR(S): Ingrosso, Loredana [Reprint author]; Vetrugno, Vito

[Reprint author]; Cardone, Franco [Reprint author];

Pocchiari, Maurizio [Reprint author]

CORPORATE SOURCE: Laboratory of Virology, Istituto Superiore di Sanita, Viale

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SOURCE: Trends in Molecular Medicine, (June, 2002) Vol. 8, No. 6,

pp. 273-280. print.

ISSN: 1471-4914.

DOCUMENT TYPE: Article

General Review; (Literature Review)

LANGUAGE: English

ENTRY DATE: Entered STN: 26 Jun 2002

Last Updated on STN: 26 Jun 2002

Clinical criteria for the diagnosis of sporadic, iatrogenic and variant Creutzfeldt-Jakob diseases are now available and show an excellent sensitivity and specificity (apprx98%). Post-mortem diagnosis, based upon the identification in the brain of the pathological conformer of the prion protein (PrPSc), is also very accurate, and several diagnostic kits are now available that facilitate the immunochemical measurement of PrPSc. Several new molecular diagnostic techniques aimed at increasing the sensitivity and specificity of PrPSc detection, and at identifying markers of disease that are other than PrPSc, are the subject of ongoing studies. The aim of these studies is to develop preclinical screening tests for the identification of infected, but still healthy, individuals. These tests are also badly needed to check the safety of blood or blood-derived products, and to ensure meat safety in European countries.

L8 ANSWER 16 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on

STN

ACCESSION NUMBER: 2001:133509 BIOSIS DOCUMENT NUMBER: PREV200100133509

TITLE: Putting prions into focus: Application of single molecule

detection to the diagnosis of prion diseases.

AUTHOR(S): Giese, A.; Bieschke, J.; Eigen, M.; Kretzschmar, H. A.

[Reprint author]

CORPORATE SOURCE: Department of Neuropathology, University of Munich,

Marchioninistr. 17, D-81377, Muenchen, Germany

SOURCE: Archives of Virology Supplement, (2000) No. 16, pp.

161-171. print.

CODEN: AVISE9. ISSN: 0939-1983.

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 14 Mar 2001

Last Updated on STN: 15 Feb 2002

AB Prion diseases are characterized by the cerebral deposition of an aggregated pathological isoform of the prion protein (PrPSc) which constitutes the principal component of the transmissible agent termed prion. In order to develop a highly sensitive method for the detection of PrPSc aggregates in biological samples such as cerebrospinal fluid (CSF), we used a method based on Fluorescence Correlation Spectroscopy (FCS), a technique which allows detection of single fluorescently labeled molecules in solution. Within the FCS setup, fluorescent photons emitted by molecules passing an open volume element defined by the beam of an excitation laser focussed into a diffraction-limited spot are imaged confocally onto a single photon counting detector. Aggregates of PrPSc could be labeled by co-aggregation of probe molecules such as monomeric recombinant PrP or PrP-specific antibodies tagged with a fluorescent dye. In addition to slow diffusion,

labeled aggregates are characterized by high fluorescence intensity, which allows detection and quantification by analysis of fluorescence intensity distribution. To improve detection of rare target particles, the accessible volume element was increased by scanning for intensely fluorescent targets (SIFT). To further improve sensitivity and specificity, two different probes were used simultaneously in a two-color setup. In a diagnostic model system of CSF spiked with purified prion rods, dual-color SIFT was more sensitive than Western blot analysis. In addition, a PrPSc-specific signal was also detected in a number of CSF samples derived from CJD patients but not in controls.

L8 ANSWER 17 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:193365 BIOSIS DOCUMENT NUMBER: PREV200000193365

TITLE: Rapid and reproducible quantification of hepatitis C virus

cDNA by fluorescence correlation

spectroscopy.

AUTHOR(S): Weiner, Olaf H.; Alt, Michael; Duerr, Ralf; Noegel,

Angelika A.; Caselmann, Wolfgang H. [Reprint author]

CORPORATE SOURCE: Department of Medicine I, University of Bonn,

Sigmund-Freud-Strasse 25, D-53105, Bonn, Germany

Digestion, (2000) Vol. 61, No. 2, pp. 84-89. print.

CODEN: DIGEBW. ISSN: 0012-2823.

DOCUMENT TYPE: Article LANGUAGE: English

SOURCE:

ENTRY DATE: Entered STN: 17 May 2000

Last Updated on STN: 4 Jan 2002

AB Background/Aims: Standard methods for hepatitis C virus (HCV) RNA quantification are time-consuming and often hampered by low sensitivity. Therefore, we aimed to test whether fluorescence correlation spectroscopy (FCS) could be used to read out HCV polymerase chain reactions (PCR). Methods: A single-step reverse transcriptase (RT) PCR system was adjusted to the clinically relevant range of 1 X 103 to 5 X 106 HCV cDNA copies/ml serum. Unpurified amplification mixtures were analyzed by FCS and controlled by HPLC analysis. Results: The outcome of HCV RNA quantitation was nearly identical no matter whether FCS or HPLC techniques were used. FCS-generated standard curves displayed sufficient linearity to allow reproducible determinations. The intraserial variation of cDNA quantification after PCR amplification was +- 3.2%, the interserial variation +- 4.3%. Repeated quantifications of HCV genotype 1b RNA from the sera of 8 patients revealed titers from 1 X 104-5 X 106 genome equivalents/ml. The results correlated significantly (r = 0.755; p =0.03) with a widely used commercially available assay. Conclusion: FCS may become a useful tool for rapid and reproducible HCV RNA quantification in the future.

L8 ANSWER 18 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:535124 BIOSIS DOCUMENT NUMBER: PREV199799834327

TITLE: Fluorescence correlation spectroscopy

for rapid and sensitive HCV RNA quantification.

AUTHOR(S): Weiner, O. H. [Reprint author]; Alt, M.; Noegel, A. A.

[Reprint author]; Caselmann, W. H.

CORPORATE SOURCE: Dep. Cell Biol., Univ. Bonn, Bonn, Germany

SOURCE: Hepatology, (1997) Vol. 26, No. 4 PART 2, pp. 141A.

Meeting Info.: 48th Annual Meeting of the American Association for the Study of Liver Diseases. Chicago,

Illinois, USA. November 7-11, 1997. CODEN: HPTLD9. ISSN: 0270-9139.

DOCUMENT TYPE: Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)

LANGUAGE:

English

ENTRY DATE:

Entered STN: 12 Dec 1997

Last Updated on STN: 12 Dec 1997

ANSWER 19 OF 21 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on L8

ACCESSION NUMBER: 1995:432046 BIOSIS DOCUMENT NUMBER: PREV199598446346

Fluorescence correlations, single molecule detection and TITLE:

large number screening. Applications in biotechnology.

AUTHOR(S): Rigler, Rudolf

Dep. Med. Biophysics, Karolinska Inst., S-171 77 Stocholm, CORPORATE SOURCE:

Sweden

Journal of Biotechnology, (1995) Vol. 41, No. 2-3, pp. SOURCE:

177-186.

CODEN: JBITD4. ISSN: 0168-1656.

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 10 Oct 1995

Last Updated on STN: 10 Oct 1995

Fluorescence correlation spectroscopy (FCS), when AB

carried out under conditions with low background as obtained in very small volume elements, is a powerful tool for examining molecular interactions as well as their time dependence. Interactions of biological importance which can be analyzed are hybridization between nucleic acid primers and DNA or RNA targets, between peptide ligands and isolated as well as cell-bound receptors, between antigen and antibodies. Since the interaction can be analyzed rapidly in small volumes without the need for separating unbound from bound ligand, an important application of FCS is envisaged in large-scale drug screening. The sensitivity has been advanced to the point that detection of single dye molecules is possible in the submillisecend range. This opens up the possibility for detecting rare events such as the appearance of pathogens in the early phase of infection or mutants exhibiting unusual properties when screening combinatorial libraries.

ANSWER 20 OF 21 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1314359 CAPLUS ACCESSION NUMBER:

DOCUMENT NUMBER: 144:35320

Antibody and fluorescently labeled antibody fragment TITLE:

specific to different epitopes of same antigen for

immunodetection of prion or food antigen

Kinjo, Masataka; Horiuchi, Motohiro; Fujii, Fumihiko; INVENTOR(S):

Sakata, Hiroshi; Tamura, Mamoru; Ueno, Masayoshi;

Yanagiya, Takayuki

PATENT ASSIGNEE(S): Japan Science and Technology Agency, Japan; Fujirebio

Inc.

Patent

SOURCE: PCT Int. Appl., 29 pp.

CODEN: PIXXD2

DOCUMENT TYPE:

Japanese LANGUAGE:

FAMILY ACC. NUM. COUNT:

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005119256	A1	20051215	WO 2005-JP10043	20050601
W: AE, AG,	AL, AM, AT	, AU, AZ,	BA, BB, BG, BR, BW, BY,	BZ, CA, CH,
CN, CO,	CR, CU, CZ	DE, DK,	DM, DZ, EC, EE, EG, ES,	FI, GB, GD,
GE, GH,	GM, HR, HU	, ID, IL,	IN, IS, KE, KG, KM, KP,	KR, KZ, LC,

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LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG,
            NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL,
             SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA,
             ZM, ZW
         RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM,
            AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,
             EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT,
             RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML,
            MR, NE, SN, TD, TG
     JP 2005345311
                          A2
                                20051215
                                            JP 2004-166440
PRIORITY APPLN. INFO.:
                                            JP 2004-166440
                                                                A 20040603
    It is intended to provide a detection and/or assay method whereby an
    abnormal prion or an antigen, for example, an antigenic protein such as a
    harmful protein contained in a food material can be quickly and accurately
    detected and/or assayed by a convenient procedure. In detecting or
    assaying an antigen mol. by using the fluorescence
     correlation spectrometry (FCS), a fluorescence-labeled antibody
     fragment and a non-fluorescence-labeled complete antibody capable of
    binding to the fluorescence-labeled antibody fragment via an antigen are
     employed. Thus, a significant difference in diffusion speed arises
    between the fluorescence-labeled antibody fragment not bonded to the
    antigen and a complex formed by the antigen/antibody reaction among the
     fluorescence-labeled antibody fragment, the antigen and the
    non-fluorescence-labeled complete antibody. According to this method, an
    antigen can be detected and assayed by using FCS, even in the case of an
    antigen with a relatively low mol. weight such as an antigenic protein,
     independently from the shape or mol. weight of the antigen and thus antigens
     over a wide scope can be quickly assayed.
REFERENCE COUNT:
                         17
                               THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS
                               RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT
    ANSWER 21 OF 21 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
ACCESSION NUMBER:
                      2001-168568 [17]
                                         WPIDS
DOC. NO. CPI:
                      C2001-050389
                      Species-specific detection of organisms, particularly for
TITLE:
                      identifying bacteria causing sepsis, comprises
                      amplification and elongation of ribosomal nucleic acid.
DERWENT CLASS:
                      B04 D13 D15 D16
                      KRUPP, G; SCHEINERT, P; SOELLER, R; SPENGLER, U
INVENTOR(S):
PATENT ASSIGNEE(S):
                      (ARTU-N) ARTUS GES MOLEKULARBIOLOGISCHE DIAGNOSTI
COUNTRY COUNT:
                      87
PATENT INFORMATION:
    PATENT NO
                     KIND DATE
                                   WEEK
                                             LА
                                                  PG
                    A1 20010201 (200117)* GE
    WO 2001007648
        RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
            OA PT SD SE SL SZ UG ZW
        W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB
            GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU
            LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR
            TT UA UG US UZ VN YU ZA ZW
    AU 9953710
                     A 20010213 (200128)
    EP 1198597
                     A1 20020424 (200235)
                                          GE
         R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
            RO SE SI
APPLICATION DETAILS:
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PATENT NO	KIND	APPLICATION	DATE
WO 2001007648	Δ1	WO 1999-EP5234	19990722

AU 9953710	Α	AU 1999-53710	19990722
		WO 1999-EP5234	19990722
EP 1198597	A1	EP 1999-939394	19990722
		WO 1999-EP5234	19990722

## FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9953710	A Based on	WO 2001007648
EP 1198597	Al Based on	WO 2001007648

PRIORITY APPLN. INFO: WO 1999-EP5234 19990722

AN 2001-168568 [17] WPIDS

AB WO 200107648 A UPAB: 20010328

NOVELTY - Species-specific detection of prokaryotic and eukaryotic organisms comprising nucleic acid amplification, is new.

DETAILED DESCRIPTION - Species-specific detection of prokaryotic and eukaryotic organisms by nucleic acid amplification, comprises:

- (a) amplifying a region of DNA flanked by conserved sequences using primers that contain the sequences;
- (b) treating the amplicon with a sequencing primer (SP) that hybridizes to a sequence that is conserved within the organisms being tested for;
- (c) performing a chain-breaking polymerization reaction, using only 3 of the 4 deoxynucleotide triphosphates (dNTP) or 3 dNTP and a chain-breaking dNTP, where SP will generate different elongation products (E) from each test organism;
  - (d) determining the lengths of E; and
- (e) identifying the organism by comparing the sequences of E with known sequences.

Optionally one or more similar analyzes are done with different SP to generate a pattern of E that is used to characterize organisms.

INDEPENDENT CLAIMS are also included for the following:

- processing and/or concentrating bacteria by lysis of non-bacterial cells then centrifuging to produce a bacterial pellet;
- (2) isolating and/or enriching bacterial DNA by lyzing bacteria in the pellet of (1);
  - (3) kits for performing methods (1) and (2); and
  - (4) kit for performing the new detection process.
  - USE The method is especially used to detect:
  - (i) sepsis-causing bacteria in blood;
  - (ii) protozoa (flagellates, ameba or ciliates) in blood;

Salmonella in meat; and

(iii) fish in spawn.

Other applications include:

- (i) detecting contaminants in food, seeds etc.;
- (ii) detecting helminths, or fungal pathogens (in humans, animals or plants);
- (iii) identification of varieties, races etc. in animal and plant breeding;
  - (iv) investigation of biodiversity; and
- (v) (diagnostic) detection of infectious agents in a wide range of tissues.

ADVANTAGE - The method is easy to perform, suitable for routine clinical use, and is highly specific for a very wide range of pathogen types, with simple and inexpensive differentiation between individual species within a type. It can detect even very small concentrations of microorganisms and can be applied to almost any sort of sample.

Dwg.0/0

(FILE 'HOME' ENTERED AT 15:13:36 ON 13 JUL 2006)

FILE 'MEDLINE, BIOSIS, CAPLUS, SCISEARCH, EMBASE, WPIDS' ENTERED AT 15:19:09 ON 13 JUL 2006

L1	6474	(FLUORESCEN?	(S)	FLUCTUAT?)	OR	FLUORESCENCE	(W)	CORRELATION
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L2 27 PATHOGEN AND L1

L3 22 DUP REM L2 (5 DUPLICATES REMOVED)

L4 14 PY>2002 AND L3 E WOLF DAVID?/AU

E WOLF DAVID E?/AU

L5 73 E2 OR E4

L6 0 D5 AND L1

L7 7 L5 AND L1 L8 21 L3 NOT L7

=> FIL STNGUIDE

COST IN U.S. DOLLARS
SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST
106.59
108.48

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE
ENTRY
SESSION
CA SUBSCRIBER PRICE

-3.75

-3.75

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AND TECHNOLOGY CORPORATION, AND FACHINFORMATIONSZENTRUM KARLSRUHE

FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Jul 7, 2006 (20060707/UP).

=> logoff hold

COST IN U.S. DOLLARS SINCE FILE TOTAL SESSION ENTRY FULL ESTIMATED COST 1.14 109.62 DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) SINCE FILE TOTAL ENTRY SESSION -3.75 0.00 CA SUBSCRIBER PRICE

SESSION WILL BE HELD FOR 60 MINUTES STN INTERNATIONAL SESSION SUSPENDED AT 15:39:09 ON 13 JUL 2006